U.S. National Institute of Health
Bulletin No. 1.

BULLETIN OF THE HYGIENIC LABORATORY,
U. S. Marine-Hospital Service.
WALTER WYMAN, *Surgeon-General.*

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PRELIMINARY NOTE

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Viability of the Bacillus Pestis

(FROM PUBLIC HEALTH REPORTS, MAY 25, 1900).

M. J. ROSENAU,

PASSED ASSISTANT SURGEON, DIRECTOR HYGIENIC LABORATORY,
U. S. MARINE-HOSPITAL SERVICE,
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PRELIMINARY NOTE

ON THE

VIABILITY OF THE BACILLUS PESTIS.

M. J. Rosenau, Passed Assistant Surgeon, and Director of the Hygienic Laboratory, United States Marine-Hospital Service.

There seem to be three factors that influence the life of the bacillus pestis in the outer world, viz, light, moisture, and temperature. The bacillus withstands quick drying very badly, as all the experiments in this direction indicate. It can not live long in the sunshine. High temperatures are invariably fatal.

That it always and under all circumstances dies in so short a time as five days, as the work of Kitasato and Wilm first indicated, must now be doubted in view of the experience of Abel, Ficker, Batzaroff, Hankin, The German Plague Commission, Germano, Giaxa, and myself.

The bacillus of plague does not exist in nature on glass cover slips, nor yet in the desiccator over concentrated sulphuric acid. With us it certainly would not be exposed to drying in our houses, and on fabrics, at temperatures of 30° and 37° C., conditions under which many of the tests to determine the viability of the organism were made.

We ought, therefore, not to apply the experience of the laboratory too literally to the life history of the plague bacillus in nature, for we can not imitate all the conditions under which the organism may exist. We may determine with fair certainty the length of time the plague bacillus may live under given conditions; and in general terms we can state whether it is a hardy organism, resistant to influences usually detrimental to bacterial life, or one that loses its virulence and dies quickly when removed from its natural habitat.

In this laboratory we have worked with the organism from 5 sources, 1 from Djiddah, 1 from Oporto, 1 from Rio de Janeiro, 1 from Bombay, and 1 from the New York quarantine case. We find that all these 5 races thrive well under the usual laboratory conditions on the ordinary media.

They can in no sense be considered tender organisms, as was at first supposed. They are much easier to cultivate than the lanceolate coccus of pneumonia or the streptococci. In fact, they resemble more closely the hardier of the hemorrhagic septicæmic group.

In this connection, Batzaroff (*a*) states: "It may be said that it is a mistake to consider the microbe of human pest to be very frail. There undoubtedly exist varieties that attenuate very quickly, and die outside the living body in a relatively short time. There are other varieties that retain their viability under similar conditions a long time, and continue to live in artificial media for months and even years without notably losing virulence. We have seen that certain of our cultures which have been kept in the laboratory without any precautions and exposed to the light for three and one-half months, still kill animals when injected hypodermatically with but slight retardation; and that regenerated 2 or 3 times they almost regain the virulence they first possessed. It is not always easy to obtain an attenuated culture of pest. It requires much time and a combination of various artifices."

On first glance over the literature on the viability of the organism there appears to be an irreconcilable difference among the various observers as to the length of time the organism will live and maintain its virulence outside the body, but on closer study it becomes apparent that these differences are due to different conditions under which the experiments were carried out, especially differences in temperature. We must not lose sight of the fact that some races of the organism are more hardy than others.

It is the experience of all observers that the bacillus can not live long outside the body when dried at a temperature of 30° C., or over, but at a temperature lower than this and under 20° C., it has been kept alive sixty and seventy-five days.

The German Plague Commission (*b*) found that the organism always lost its power of infection when dried, within eight days, in India, but after returning to Germany could be kept alive after drying twenty-eight days, at 15° to 18° C.

My own experience indicates that the organism, when dried, will die quickly if the temperature reaches 27° C. but that at 23° C. and under it may live a long time.

A series of experiments has been undertaken in this laboratory and a few of the results are given in advance showing how long the organism may live and retain its virulence when dried under various conditions.

ALBUMIN-GELATIN BALLS INFECTED WITH PLAGUE CULTURE.

A little ball of sterile absorbent cotton about the size of a pea is soaked with a few drops of a gelatin culture of plague (Djiddah) mixed with egg albumin, and exposed in a Petri dish in the photographic dark room (20° to 23° C.) and the cool chamber (17° to 19° C.).

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a Batzaroff La Pneumonie pesteuse experimentale. Annales de l'Institut Pasteur, May, 1899, page 391.

b Kaiserliche Gesundheitsamt, Bd. 16, Berlin 1899, page 274, *et seq.*

The little balls soon dry and the gelatin-albumin shrinks to a flaky, dry, hard mass.

From time to time one of the dried balls is taken out, planted in bouillon, and incubated. In case a growth appears the organism is tested on all the media, and on mice.

It was found that the organism lived and remained virulent for mice under these conditions for seventy-five days in the dark room at about 20° to 23° C. and for seventy-five days in the cool chamber at 17° to 19° C. Whether it may live longer will be determined from time to time and reported later on.

CRASH INFECTED WITH PLAGUE CULTURE.

In another series of experiments little squares of fabric (crash) were sterilized and inoculated with a three-day-old bouillon culture of the bacillus pestis. One set of these was allowed to dry out in Petri dishes in a dark corner of the laboratory where the temperature ranges from about 20° to 27° C., another set in the cool chamber (17° to 19° C.), and another set in the photographic dark room (20° to 23° C.). The squares were removed at intervals and planted in bouillon and in case a growth appeared it was studied for purity and pathogenicity in media and mice.

The following are some of the results:

Bouillon culture on crash.

Time.	Laboratory 20° to 27° C.,	Cool chamber, 17° to 19° C.	Dark room, 20° to 23° C.
4 days	+	+	+
8 days	—	+	+
11 days	+	—	—
13 days	+	+	+
15 days	—	—	+
21 days	—	+ Killed mouse in two days.	+ Killed mouse in two days.
26 days	—	—	+ Killed mouse in three days.
35 days	—	+	+
48 days	—	+ Killed mouse in three days.	+ Killed mouse in two days.

From this table it appears that the organism died after thirteen days when kept at a temperature which occasionally rose to 27° C.

At a somewhat lower temperature it remained alive and virulent forty-eight days. Whether longer, will be reported upon the completion of the work. This table indicates plainly how sensitive this particular organism is to a very slight difference of temperature.

PINE WOOD INFECTED WITH PLAGUE CULTURE.

Another series of tests was made with splinters of pine wood about the size of a match stick. They were sterilized and soaked in a three-day-old bouillon culture of plague and then placed in Petri dishes

which were kept in the laboratory (20° to 27° C.), cool chamber (17° to 19° C.), and the dark room (20° to 23° C.), with the following results:

Pieces of pine wood inoculated with bouillon culture of bacillus pestis.

Time.	Laboratory.	Cool chamber.	Dark room.	Time.	Laboratory.	Cool chamber.	Dark room.
4 days	+	+	+	13 days	—	—	—
8 days	—	+	+	18 days	—	—	—
11 days	—	—	+	21 days	—	—	—

The same culture was used to impregnate the pieces of pine wood as was used for the squares of crash in the preceding table, and these two objects thus infected were exposed to precisely the same conditions. It may therefore be assumed that the organism lives a shorter time on the one than on the other.

PAPER INFECTED WITH PLAGUE CULTURE.

Another series of tests was made with pieces of filter paper and pieces of glazed (sized) paper. This paper is cut into little squares and sterilized and impregnated in the usual way with a three-day-old bouillon culture of the organism. These pieces were placed in Petri dishes and kept in the desk in my office where the temperature ranges from 20° to 27° C. The results follow:

Plague culture dried on paper.

Time.	Filter paper.	Glazed paper.	Time.	Filter paper.	Glazed paper.
4 days	+	+	13 days	—	—
8 days	+	+	18 days	—	—
11 days	—	—			

On account of the importance of this subject, a summary of the literature follows:

KITASATO (*a*) found the organism alive after four days when dried on a glass cover slip and kept at 28° to 30° C. It grew after one to thirty-six hours, but not after four days. He used the pus of buboes from man. His experiments with serum cultures gave similar results. He was the first to study this question.

WILM (*b*) found the organism alive after four and one-half days on a glass slip. He exposed pure cultures at a temperature of 29° to 31° C. He could obtain no growth in bouillon after an exposure of four and one-half days. In vacuo, the bacilli were killed after an exposure of three hours.

a Preliminary notice of the bacillus of bubonic plague, Hongkong, July 7, 1894.
b Uber die Pestepidemic in Hongkong im Jahre 1896. Hygienische Rundschau, 1897, Bd. VII, page 290.

R. ABEL (*a*) used pure cultures from bouillon, agar-agar and blood serum, or pus from the peritoneal cavity of guinea pigs, containing the bacillus pestis. These were planted on various objects—glass cover slips, silk, wool, woolen cloth, linen, and pieces of ox skin, and in sterile earth. Also pieces of the organs, liver, and spleen of animals dead of plague were dried. Approximately similar sizes were taken every day from each object. The result of these experiments showed that the manner of drying influenced the viability of the pest bacillus. No matter on what material, the result of drying at the temperature of 35° C. in the incubator, or 20° C. in vacuo over sulphuric acid, the bacillus was always found dead in two, or at most, four days. If the drying was permitted at the room temperature, 16° to 20° C. in a dark place, the bacilli remained alive much longer and the material in which they were dried markedly influenced the result.

On cover slips they remained alive six to nine days, from pus and cultures. Only once in four tests were the bacilli found alive a longer time—fourteen days—but not longer than this.

On threads of various sorts, on linen, and in pieces of organs, the bacilli were found alive after thirty days; if longer, it could not be determined, because the work was interrupted. In the longer exposures the threads planted in bouillon and kept undisturbed showed the outgrowth of isolated colonies here and there, indicating that many of the bacilli had lost their power of multiplication.

He, therefore, concluded that forced drying at a temperature over 30° C. or by substances that have a high affinity for water such as concentrated sulphuric acid, the bacillus pestis dies quickly. Slower drying at lower temperature is less harmful. However, even at room temperature the quicker drying, such as on a cover glass, is more harmful to the bacillus than the slower drying on pieces of cloth or in pieces of tissue.

"The fact that the bacillus remains alive longer when dried at 16° to 20° C. than was at first announced, teaches that in our climate (Hamburg) at least, we can not expect the organism to quickly disappear in clothing, etc."

On fresh skin the bacillus was found alive after ten days. These tests were not altogether satisfactory because the sterilizing of the skin before infecting it with pest was not successful after washing and eight days' immersion in ether and alcohol. It is possible that the bacilli are alive after ten days, though not demonstrable on account of the growth of other organisms.

Further tests are being made with cultures, and the bacillus in blood and organs placed on various objects, and kept under various conditions, report on which will be submitted later.

MARTIN FICKER (*b*) tested the effect of drying and moisture alter-

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a Zur Kenntnis des Pestbacillus. Centralblat für Bakteriologie und Parasitenkunde, etc., Bd. XXI, 1897, page 497.

b Zeitschrift fur Hygiene, Bd. XXXIX, 1898, page 25.

nately applied on the viability of the plague bacillus and found it to be more harmful than simple drying. He found that changing the conditions from dryness to moisture caused the death of the organism in twenty to twenty-eight hours, while the same in the desiccator lived eight or nine days.

He used twenty-four hour old cultures from agar-agar plates and made thin spreads on cover glasses by means of the platinum œse. Moisture was added twice daily. The cover glass was planted in bouillon and in case growth appeared this was plated out on gelatin.

	Alternate moisture.		Desiccator.	
	†	O	+	O
	Hours.	*Hours.*	*Days.*	*Days.*
Culture 24 hours	20	28	8	9
Do	24	36	9	11
Culture 36 hours	36	48	11	12

This demonstrates that cultures die more rapidly in drying when moisture and dryness are alternated.

BATZAROFF (*a*) has been able to demonstrate that even in the dry state the pest organism remains living and virulent a long time. The organs of an animal dead of pest, as well as cultures mixed with infusorial earth, are subjected to desiccation in vacuo at "room temperature."

From time to time a piece of the dried substance is tested by grinding in a sterilized mortar, and the powder thus obtained is introduced in small amounts into the nose of an animal. The results of these experiments, which are indicated in the subjoined table, show that the virus of pest supports desiccation for a long time very well when it is in an albuminous medium, as the pulp of the spleen or any other organ. Under such conditions it attenuates very slowly; in fact, in the first two or three weeks scarcely at all. On the other hand, if it is not protected, as in the case of dried cultures in infusorial earth, its virulence diminishes rapidly, so that in three weeks, inoculated into the nose of animals, the dried cultures produced no morbid effect.

Plague-pneumonia produced by nasal inoculations of dried virus.

Splenic pulp.		Dried cultures in infusorial earth.	
Duration of desiccation.	Duration of sickness.	Duration of desiccation.	Duration of sickness.
	Days.		*Days.*
5 days	4½	2 days	7
8 days	4	5 days	6½
12 days	4½	7 days	(*b*)
14 days	5	19 days	12
20 days	4½	31 days	(*b*)
26 days	4½		
30 days	6½		
33 days	6½		
38 days	6½		
44 days	(*b*)		

a La Pneumonie pesteuse experimentale. Annales de l'Institut Pasteur, May, 1899, page 392.
b Resisted.

HANKIN (*a*) states that as far as his researches go they tend to show that the bubonic microbe, whether derived from cultures or the organs of deceased animals, and whether placed in cotton, or sheep's wool, or gunny cloth, uniformly die out in six days. He states, however, that owing to the fact that epidemiological evidence tends to show that clothing may in rare cases convey the infection he is unwilling to draw any definite conclusions from his researches at present.

THE GERMAN PLAGUE COMMISSION (*b*) (Gaffky, Sticker, Pfeiffer, Dieudonné) used the organism from agar-agar, and bouillon cultures; also sputum from plague-pneumonia, peritoneal exudate from guinea pigs, etc. This infectious material was placed on various objects, as glass, silk threads, filter paper, fabrics, earth, etc., and permitted to dry. The plague-infected objects were placed in Petri dishes and put in a dark corner of the laboratory to dry out. As a rule, the infected pieces were turned over from time to time to hasten the drying.

The temperature of the laboratory during these experiments was about 29° to 31° C. The objects were well protected from the light. Some were kept in an improvised ice box, in which the temperature was about 22° C. and the air saturated with moisture. The proof that the organism was still alive could, unfortunately, only be made by inoculation of mice and not by means of cultures. Small pieces of the infected objects were taken from time to time and placed under the skin of a mouse. The following are the results:

Infected with pure culture.

Object.	Temperature.	Longest time in days the organism lived.
Glass	30° to 32° C.	3
Glass in desiccator	do	1
Filter paper	do	3
Filter paper in desiccator	do	1
Silk thread	do	5
Silk thread in desiccator	do	1
Piece of silk	do	6
Piece of silk in desiccator	do	1
Piece of wool	do	6
Piece of wool in desiccator	do	2
Large piece of linen	do	7

Infected with plague organs.

Glass	30° to 32° C.	2
	22° to 24° C.	2
Filter paper	30° to 32° C.	2
	22° to 24° C.	2
Silk thread	30° to 32° C.	2
	22° to 24° C.	2
Piece of wool	30° to 32° C.	6
	22° to 24° C.	6
Large piece of gauze	30° to 32° C.	6
	22° to 24° C.	6
Large piece of linen	30° to 32° C.	6
	22° to 24° C.	8

a The plague in India, 1896, 1897, Vol. II, appendices I to T, page 10, *et seq.*

b Deutsche Pestkommission. Kaiserliche Gesundheitsamt, Bd. 16. Berlin, 1899, page 274, *et seq.*

Sputum from plague-pneumonia.

Object.	Temperature.	Longest time in days the organism lived.
Glass	30° to 32° C.	1
Silk thread	do	3
Piece of wool	do	6
Pus from bubo.		
Glass	30° to 32° C.	6
Silk thread	do	1
Piece of wool	do	1
Peritoneal exudate from plague-infected guinea pig.		
Silk thread	29° to 31° C.	
Filter paper	do	
Piece of silk	do	(*a*)
Piece of wool	do	
Glass tube	do	(*b*)

a Still virulent on the second day. *b* Dead on the seventh day.

The longest observation made by the commission on the viability of the plague bacillus was therefore eight days.

It made no difference whether cultures one to two days' old, or eight-day-old cultures were used, as far as the resistance to drying was concerned.

Pieces of the skin of mice dead of plague were also kept and it was found that they had lost their infecting power after four and six days. In dry organs the pest bacillus soon died. The longest time observed was in pieces of liver kept in sealed glass tubes. Here they lived seven days; after seven days no result was obtained.

Sterilized feces were inoculated with a bouillon culture of plague obtained fresh from a corpse and then saturated with silk threads. wool, and silk fabrics. After drying they were wrapped in sterile filter paper and, inclosed in cotton, were packed in a box and kept in the laboratory at about 29° C. After four days the pest bacillus was alive on all the test objects; after six days only in the wool, and after eight days dead in all.

The energetic drying in the desiccator over sulphuric acid hastened the death of the organism.

On account of the great importance of this question, several more tests were made, at lower temperatures, by the committee after its return to Germany. It was there possible, on account of the more favorable laboratory conditions, to make cultures as well as inoculations into mice in order to establish the life and death of the bacillus. The bacillus was kept alive in a room at 15° to 18° C. for twenty-eight days. After thirty-three days no more growth was obtained. Even after twenty-four days the growth was very sparse. The pathogenicity for mice died out much quicker after eighteen days.

"Apparently then the bacillus pestis is very sensitive to rapid and energetic drying as occurs by higher temperatures and in the desiccator.

"Slower drying at lower temperatures is much less deadly. In the climate of Germany, therefore, a rapid death of the organism in fabrics, etc., is not to be expected, as occurs in the tropics. In the hot countries no live bacilli were found in the dust."

EDUARDO GERMANO (*a*) made many observations to determine this question. He worked with a culture from the Breslauer Hygienic Institute obtained through Kral, and which had been grown through many generations in vitro. He used bouillon suspensions of agar cultures and exposed the infected objects in Petri dishes at room temperature, 16° to 20° C.

Room dust mixed with an emulsion of plague bacilli.

Time.	Moist.	Dry.	Dried over sulphuric acid.
1 day	—	—	—

Fine sand mixed with emulsion of plague bacilli.

Time.	Moist.	Dry.	Dried over sulphuric acid.	Time.	Moist.	Dry.	Dried over sulphuric acid.
1 day	+	(*a*)+	—	20 days	+	—	—
5 days	+	—	—	25 days	+	—	—
5 days	+	—	—	30 days	+	—	—
8 days	+	—	—	40 days	+	—	—
12 days	+	—	—	50 days	+	—	—
16 days	+	—	—	60 days	(*b*) +	—	—

a The dust is not yet dry.
b The number of colonies is diminished at least to the $\frac{1}{10}$ part of the original.

Loam (Humus boden) mixed with emulsion of plague bacillus.

Time.	Moist.	Dry.	Over H_2SO_4.	Remarks.
1 day	+	—	—	
3 days	+			
5 days	+			
8 days	+			
12 days	+			
16 days	+			
20 days	+			
25 days	+			
30 days	+			
40 days	+			
50 days	+			
60 days	+			

a Die Uebertragung von Infectionskrankheiten durch die Luft. Dr. Eduardo Germano. Zeitschrift fur Hygiene, Vol. XXVI, 1897; 281.

Tufa (Tuff boden) rubbed up with plague bacillus.

Time.	Moist.	Dry.	Over H_2SO_4	Remarks.
1 day	+	—	—	
5 days	+			
8 days	+			
12 days	+			
16 days	+			
20 days	+			
25 days	+			
30 days	+			
40 days	+			
50 days	+			
60 days	+			

Marly loam (Löss) rubbed up with plague bacillus.

Time.	Moist.	Dry.	Over H_2SO_4.	Remarks.
1 day	+	—	—	
3 days	+			
5 days	+			
8 days	+			
12 days	+			
16 days	+			
20 days	+			
25 days	+			
30 days	+			
40 days	+			
50 days	+			
60 days	+			

Brick dust mixed up with plague bacillus.

Time.	Moist.	Dry.	Over H_2SO_4.	Remarks.
1 day	+	+	—	
3 days	+	—		
5 days	+			
8 days	+			
12 days	+			
16 days	+			
20 days	+			
25 days	+			
30 days	+			
40 days	+			
50 days	+			
60 days	+			

From these tables it follows that the bacillus of plague withstands drying badly, while it remains alive a long time in moist conditions. Only in room dust did it die quickly under moist conditions. After two months, even under moist conditions, the number of bacteria was very much diminished. In addition to the test with dust, etc., a series was made with fabrics.

Square pieces of linen, wool, silk and filter paper (1 cm. square) were sterilized and saturated with a bouillon emulsion of culture. They were placed in Petri dishes and were exposed in the desiccator and in the room. Those that were to remain moist were placed in test tubes in the moist chamber.

The results were as follows:

Linen.

Time.	Moist.	Dry.	Over H_2SO_4.	Time.	Moist.	Dry.	Over H_2SO_4.
1 day	+	+		20 days	+		
3 days	+			25 days	+		
4 days		○		30 days	+		
7 days	+			40 days	+		
12 days	+			50 days	+		
16 days	+			60 days	+		

Wool.

Time.	Moist.	Dry.	Over H_2SO_4	Time.	Moist.	Dry.	Over H_2SO_4
1 day	+	+		20 days	+	+	+
3 days	+			25 days	+	+	+
4 days	+	+		30 days	+	+	
8 days	+	+		40 days	+		
12 days	+	+		50 days	+		
16 days	+	+		60 days	+		

Silk.

Time.	Moist.	Dry.	Over H_2SO_4	Time.	Moist.	Dry.	Over H_2SO_4
1 day	+	+	+	20 days	+		
3 days	+	+	+	25 days	+		
4 days		+	+	30 days	+		
8 days	+	—		40 days	+		
12 days	+		—	50 days	+		
16 days	+			60 days	+		

Filter paper.

Time.	Moist.	Dry.	Over H_2SO_4.	Time.	Moist.	Dry.	Over H_2SO_4.
1 day	+	+	+	20 days	+		
3 days	+		—	25 days	+		
4 days	+	+		30 days	+		
8 days	+			40 days	+		
12 days	+			50 days	+		
16 days	+			60 days	+		

It is seen, therefore, that the pest bacillus remains on fabrics quite a long time. In this respect it resembles the bacillus typhosus.

He draws the following conclusions: The pest bacillus has little resistance against drying. It can, therefore, not easily be carried in the dust or air. It is, therefore, not likely, if not impossible, that the disease is spread in the air. However, it may often occur that infection takes place through contact because the organism lives when not wholly dried. Therefore, infected clothing may be very dangerous.

V. DE GIAXA and B. GOSIO (*a*) made experiments in a small chamber partially ventilated and lighted, having a temperature varying from 10° to 18° C.

Strips of different fabrics, linen or wool, were infected after steam sterilization, and then suspended free or in small cabinets protected from the atmospheric dust. Some of the material was placed on large pieces of the same fabrics arranged so as to simulate a package.

The material of infection was deposited on the cloth either by rubbing with fresh agar-agar culture, in which case small particles of agar in addition to the germs adhere to the texture, or by the direct application of pus or blood from plague-infected animals.

The longest period of observation was thirty days; at the end of that time germs of plague were obtained from the pieces of cloth. In the case of pieces of cloth impregnated with pus or blood the observation was for twenty-nine days. On these the germs developed either in the open air or on cloth folded and inclosed in a box to simulate clothing packed in a trunk.

Pieces of linen thread were steeped in infected blood and exposed to desiccation in an incubator at an air circulation of 36° to 39° C.

After two, four, and five days several threads were placed in tubes of broth. Those exposed two days to desiccation developed slowly. Of those desiccated for four days, only 1 in 3 remained sterile; all those exposed for five days remained sterile.

Plague-infected blood was spread on a rabbit's skin and allowed to dry for four days. After fifteen days' exposure to a temperature varying from 12° to 16° C. (diffused light) the infected surface was scraped and the scrapings were cultivated. The plague bacillus soon developed.

As regards pathogenic action, the germ exposed to desiccation almost always showed a decided slowness in killing the animal.

EFFECT OF SUNLIGHT ON THE PLAGUE BACILLI.

ALBRECHT and BHON (*b*) exposed peritoneal exudate in a test tube (in August, in Vienna), to direct sunlight for six hours, and could not notice any marked difference in the organism.

ABEL (*c*) found that plague bacilli on cover glasses died after one hour exposure to sunlight at 30° C. (in Hamburg). Controls kept in the dark grew after six hours. Thicker spreads from agar cultures withstood sunning at 30° C., three and one-half hours.

KITASATO (*d*) found that the bacillus was killed at Hongkong in three to four hours' sunning. He used pus from buboes.

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a Richerche sul bacillo della peste bubbonica, etc., Annali d' igiene sperimentale, 1897, No. 7, page 261.

b Die Peste, Müller and Poch, page 61, Vienna, 1900.

c Zur Kenntnis des Pestbacillus. Centralblatt fur Bakteriologie und Parasitenkunde, etc., Bd. XXI, 1897.

d Preliminary notice of the bacillus of bubonic plague, Hongkong, 1894, July 7.

WILM (*a*) found them destroyed in four hours' exposure to the sun.

THE GERMAN PLAGUE COMMISSION (*b*) made tests in Bombay in this manner: The various objects (glass, silk threads, wool) were saturated with a virulent agar culture of the bacillus pestis. One-half of the objects were exposed to direct sunlight. The other half were laid away in a dark place for control.

In very thin layers on glass the bacillus was killed in one hour.

In thicker layers on glass they were pathogenic for mice after three hours but not after four hours.

In silk threads they were killed after three hours' sunning.

In wool they were virulent for mice after eight hours.

Other tests were made with well grown agar cultures placed in the sunlight. In one or two hours in the sun they still contained active organisms. After a whole day's sunning they were dead. The cultures are warmed very much in the sun; therefore, other tests were made by placing the agar tube in water and keeping in the sun one and one-half hours. At the end of the test the temperature of the water was 39° C. The bacillus was alive and virulent.

The following report of the experiments on the viability of the plague bacillus was published by S. L. RAPPOPORT, (*c*) St. Petersburg. The material used was allowed to soak in bouillon cultures of bacillus pestis in a dark closet for twenty-four hours, then exposed for successive days to all the sunlight obtainable, or to dry heat.

Temperature and time required to kill.

Material.	20 C. (68 F.)	36 C. (96. 8 F.)	60 C. (140 F.)	80 C. (176 F.)
	Days.	*Days.*	*Minutes.*	*Minutes.*
Silk thread	19 to 24	13	75	15
Note paper	10 to 17	5	30	15
Filter paper	10 to 24	7	45	15
Linen thread	9 to 13	4	30	15
Woolen thread	13 to 23	5	60	15

VIABILITY ON VARIOUS OBJECTS.

In sputum kept fluid, the GERMAN PLAGUE COMMISSION (*d*) found the bacillus of pest infectious after ten days.

HANKIN (*e*) made researches on various articles of produce to determine their susceptibility to endanger or support the bubonic microbe. He shows that the various descriptions of flours and grain usually stored in Bombay contain no trace of the bubonic microbe and also demonstrates that grain purposely infected does not permit of the existence of the microbe for more than about four days.

a Uber die Pestepidemic in Hongkong im Jahre 1896. Hygienische Rundschau, 1897. Bd. VII.

b Loc. cit., page 277.

c Quoted by Walter Wyman, Surgeon-General Marine-Hospital Service, in "The Bubonic Plague," Treasury Department Document No. 2165.

d Kaiserliche Gesundheitsamte. Bd. 16. Berlin. 1899.

e The Plague in India. 1896, 1897. Vol. II, Appendices I–IV, page 10 *et seq.*

Hankin worked with linseed, yellow rape seed, brown rape seed, tilseed or ginjelly seed, ground nuts, castor seeds, poppy seeds, wheat (new hard red), wheat (another light variety), flour.

In order to test whether the microbe was still present in a living condition in a specimen of previously infected grain his method consisted of making an extract of this grain and injecting it into a mouse.

He used agar cultures making bouillon suspensions. About a kilogram of grain to be tested was placed in a sterile stoppered foot glass. One c. c. of the bouillon emulsion of the microbe was then poured into the foot glass, care being taken that it should fall into the center of the grain. The stopper was replaced and the foot glass was immediately violently shaken for about one-quarter of an hour.

Extracts made from the grain in this manner were inoculated into mice with the following results:

Extract of the following injected into mice.	At once after infecting the grain.	Two days after infecting the grain.	Four days after infecting the grain.	Eleven days after infecting the grain.	Thirteen days after infecting the grain.
Linseed	Died within 24 hours.	Died after 7 days.	Died after 15 days.	Survived	Survived.
Yellow rape seed	Died within 72 hours.	Died after 24 hours.	Survived	do	Died after 24 hours.
Brown rape seed	Died within 48 hours.	Died after 4 days.	do	Died after 24 hours.	Survived.
Tilseed	Died within 24 hours.	Survived	do	Died after 7 days.	Do.
Ground nuts	do	Died after 11 days.	do	Died after 24 hours.	Do.
Poppy seed	do	Died after 48 hours.	do	do	Do.
Wheat (new hard red).	Died within 48 hours.	Survived	do	do	Do.
Wheat (another kind).	Survived	do	do	do	Do.
Flour	Died within 48 hours.	Died after 48 hours.	do	do	Do.

Hankin concludes from these experiments that the bubonic microbe derived from pure cultures perishes within thirteen days after being added to the above-mentioned specimens of grain and seeds.

Further experiments were made with grain and seeds by mixing the spleen of a rat and the liver, spleen, and œdema from a mouse, both animals having died after plague infection, instead of cultures of the organism. The organs were powdered up in a mortar with powdered glass and mixed with bouillon and added to the grain and seed. The further proceedings were as above.

The result of this showed that grain infected with the organs of animals dead of the plague lost its infectious power completely within six days.

Another set of experiments was then conducted by infecting the grain with sputum from a case of pneumonic plague. Sputum was taken, in which the organism was demonstrated to exist in large numbers in very virulent condition.

These also showed the plague infection to die out from all the variety of seed and grain tested within six days.

ABEL (*a*) found living bacilli of plague in water after twenty days. He added an œse to 50 c. c. of sterile distilled and tap water.

The GERMAN PLAGUE COMMISSION (*b*) found that the organism was no longer virulent after five days in tap and ten days in distilled water.

YOKOTE (*c*) worked on the problem of how long the plague bacillus can remain alive in the dead body. He used mice for his experiments because they are very susceptible. The mice were inoculated and the presence of the bacilli demonstrated in the heart's blood of the dead animals, which were then placed in a wooden casket. Each casket was covered with a lid and then buried in a metal box filled with garden earth. From time to time water was poured on the earth so that it always had a certain moisture. The temperature of the room was taken daily. After a certain time the bodies were disinterred, the amount of moisture present in the earth surrounding the casket determined quantitatively, also examined bacteriologically and by animal experiments for pest bacilli. Mice were used for this purpose, and glycerin agar for culture medium.

On disinterring the body the amount of decomposition was noted, and by means of cover-glass prepartions he determined the characters and numbers of micro-organisms present in the various organs. Plate cultures were made from the internal organs, on agar-agar, and some of the heart's blood, liver, or in case of advanced decomposition, some of the remains were inoculated into mice subcutaneously.

As a result of these experiments, he concludes that the pest bacillus loses its life and its power of infection in a relatively short time. It remains alive, at most, twenty to thirty days. This depends on the temperature. The higher the temperature, the stronger the decomposition, the shorter the life of the bacillus. In summer many saprophytes grow in the body and cause products which kill the pest bacillus. In cold winter this growth of saprophytes is less, and the pest bacillus could therefore live and maintain its virulence a longer time.

He states that his experiments are not sufficient to determine positively the length of time the pest bacillus may live in a buried body, but he gives it as his belief that the organism can not live longer in the cadaver than spore-bearing organisms because it does not have spores. It is also of interest to notice that the pest bacillus did not escape from the wooden casket into the surrounding earth. It seems, therefore, that there is no danger in the burial of pest cadavers of infecting the surrounding earth as long as the coffin is tight.

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a Loc cit. *b* Loc cit.

c Uber die Lebensdauer der Pestbacillen in der beerdigten Tierleiche. Dr. Z. Yokote. Centralblatt fur Bakteriologie und Parasitenkunde, etc., Vol. XXIII, 1898, No. 24, page 1030.

THE LENGTH OF TIME THE PEST BACILLUS WITHSTANDS DRYING.

Author.	Conditions.	Resisted.	Killed.
Kitasato	Dried on cover glass slips at 28° to 30° C.	1 to 36 hours	4 days.
Wilm	Dried on glass slips at 29° to 31° C.	4½ days	After 4½ days.
	Dried in vacuo		3 hours.
Abel	Dried on various objects at 35° C.		2 to 4 days.
	Dried on various objects in vacuo over H_2SO_4 at 20° C.		2 to 4 days.
	Dried on cover slips from pus and culture at 16° to 20° C.	14 days	After 14 days.
	Dried on threads, in linen, in tissue at 16° to 20° C.	30 days	
	Dried on piece of fresh skin, 16° to 20° C	10 days	
Ficker	Dried on cover glasses in desiccator	8 to 11 days	9 to 12 days.
	Dried and moistened alternately	20 to 36 hours	28 to 48 hours
Batzaroff	Dried splenic pulp, "room temperature."	28 days	44 days.
	Dried cultures mixed with infusorial earth.	19 days	31 days.
Hankin	Dried on sheep's wool, and gunny cloth and cotton.		6 days.
German Plague Commission.	Dried on glass, filter paper, silk threads, pieces of silk and wool and linen, at 30° to 32° C. exposed both in room and in desiccator.	7 days.	
	Dried on the same objects at 30° and 32° C., and 22° to 24° C., infected with bacillus from plague organs.	8 days.	
	Dried and kept at 15° to 18° C.	28 days	33 days.
Germano	In moist and dry room dust, 16° to 20° C.		1 day.
	Dried in fine sand and brick dust, 16° to 20° C.	1 day	5 days.
	Dried in loam, tufa, and marl		1 day.
	In moist sand, loam, tufa, marl, and brick dust at 16° to 20° C.	60 days.	
	On moist linen, wool, silk, and filter paper at 16° to 20° C.	60 days.	
	Dried on linen, 16° to 20° C.	1 day	4 days.
	Dried on wool, 16° to 20° C.	30 days.	
	Dried on silk, 16° to 20° C.	4 days	8 days.
	Dried on filter paper	4 days.	
Giaxa and Gosio	Dried on linen and wool; agar cultures, pus and blood used. 10° to 13° C.	30 days	
	Dried on linen thread, 36° to 39° C.	4 days	5 days.
	Rabbit skin infected with plague-infected blood, 12° to 16° C.	15 days	
Rosenau	Dried on little balls of gelatin-albumen, 20° to 23° C.	75 days	
	Dried on little balls of gelatin-albumen, 17° to 19° C.	75 days	
	Dried on crash, 17° to 19° C. and 20 to 23° C.	48 days	
	Dried on crash, 20° to 27° C.	13 days	15 days.
	Dried on paper, 20° to 27° C.	8 days	11 days.
	Dried on pine wood, 20° to 27° C.	4 days	8 days.
	Dried on pine wood, 17° to 19° C.	8 days	11 days.
	Dried on pine wood, 20° to 23° C.	11 days	13 days.

EFFECT OF SUNLIGHT ON PLAGUE BACILLI.

Author.	Condition.	Killed.	Withstood.
			Hours.
Abel	In Hamburg, 30 C., thin spreads on cover glasses	1 hour	
	Thicker spreads		3½
Kitasato	In Hongkong. Pus from buboes	3 to 4 hours	
Wilm		4 hours	
Albrecht and Bhon	Vienna in August, peritoneal exudate		6
German Pest Commission	In Bombay, thin layers on glass	1 hour	
	thicker layers on glass	4 hours	3
	silk threads	3 hours	
	in wool		8
	agar test tube culture	All day	2
Rappoport	In St. Petersburg, silk threads, 20 C	24 days	
	note paper, 20 C	17 days	
	filter paper, 20 C	24 days	
	linen thread, 20 C	13 days	
	woolen thread, 20 C	23 days	

VIABILITY OF THE ORGANISM IN VARIOUS MEDIA.

Author.	Condition.	Alive.	Dead.
		Days.	*Days.*
German Plague Commission	In sputum, 30° to 32° C	6 to 10	
	In pieces of liver kept in sealed tube	7	(*a*)
	In sterilized feces in thread, fabrics, and paper, 29° C.	4	8
Hankin	On seeds, grains, and flours		13
Abel	In water, distilled and tap	20	
German Plague Commission	In tap water		5
	In distilled water		10
Yokoto	In cadavers of mice, buried	20 to 30	

a After 7 days.